Ornis Fennica 95: 82–88. 2018

Brief report

A comparative laboratory study of the preservation of different rodent bones in pellets of Strigiformes

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Received 27 February 2017, accepted 30 November 2017

Studies of variation in preservation of different skeletal elements in the pellets of birds of prey helps us understand which bones should be used for effective and reliable diet analyses. The preservation of cranial and postcranial elements of skeletons of laboratory mice was studied in owl pellets. The research was conducted with three male individuals of three species: Long-eared Owl, Tawny Owl and Pygmy Owl. The owls were fed with adult and immature mice in laboratory conditions. The preservation of bones was defined as the ratio of the number of whole specimens of a certain bone to the total number of bones which were contained in the body of one mouse. The largest and the most massive bones had a high preservation. The upper part of cranium and the large bones of limb girdles had the highest preservation in the owl pellets. The skeletal elements of adult mice had the highest preservation in the pellets of Long-eared Owl. The bones of immature mice had higher preservation in the pellets of Pygmy Owl compared to Long-eared and Tawny Owl. Age differences in bone size and thickness were explained the variation in preservation of immature and adult mouse bones. Based on literature and our own data, we consider that prey identification methods based on cranial and postcranial elements complement each other. We therefore advise to use both elements together for maximum reliability of estimations of species composition and the number of prey based on pellet analysis.

1. Introduction

Interactions between birds of prey and their prey regulate their respective population sizes. Evaluation of this interaction and the study of the diet of birds of prey is one of the central issues in their ecology (Potapov 1989, Priklonskii & Ivanchev 1993). Moreover, studies of the diet composition of birds of prey allows to reveal the species composition and the abundance of prey in a certain territory. In addition it allows to identify the role of individual species of prey in the diet of a predator.

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Pellet analysis is a popular and informative method for studying food habits of raptors (Errington 1930, 1932, Mikkola 1983, Marti 1987). There are several approaches for the identification of prey in pellets which are based on different preservation of skeletal elements (Potapov 1989). Most authors consider that the most precise approach is identification based on cranial bones. Some studies show that these bones have a better preservation than other skeletal elements in the pellets of birds of prey (Potapov 1989, Priklonskii & Ivanchev 1993).

In addition, other approaches can be used for the determination of prey. Some methods are based on coxae, tibiae and femora. These bones are very species-specific (Guilday 1951, Brown & Twigg 1969, Mayakov & Shepel 1987). Also humeri of small mammals and birds preserve well and are identifiable on species-level (Mitropolsky & Mitropolsky 2009, Mitropolsky & Fundukchiev 2009). This method works properly when a predator cannot eat large prey completely and eats only the hind part (Mitropolsky & Mitropolsky 2009). Tibiae, femora and coxae can be used as additional elements in the determination of the prey while using bones of the cranium (Mayakov & Shepel, 1987).

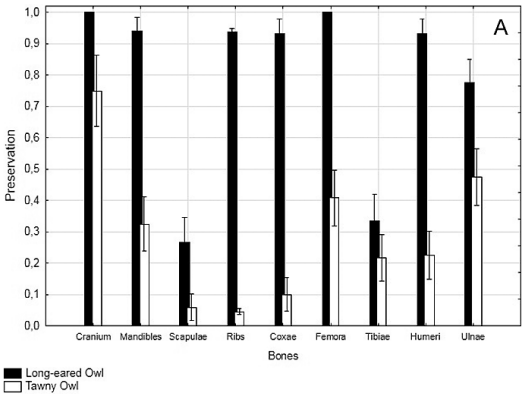
On the whole, the number of studies assessing bone preservation in pellets of birds of prey is low. Lowe (1980) showed in a laboratory study that there were not many cranial bones of mice and voles in the pellets of the Tawny Owl. The author also noted different digestibility of the cranial bones depending on the species of the prey. The most extensive study in this area is the research on the effect of digestion on the osteological composition of owl pellets by Raczyński & Ruprecht (1974). In this study the authors presented data on the preservation of the upper part of cranium, mandibles and coxae of basic species of prey in the pellets of three species of owls. In laboratory conditions the upper part of the cranium had a high preservation while the preservation of coxae was low. Unfortunately the authors did not consider the preservation of other elements of the postcranial skeleton. Moreover, the owls were fed on different species of small mammals which differ significantly in the dimensional characteristics that can greatly affect the results. The question of the most effective method for identification of prey in the pellets of birds of prey is not decided due to lack of a single point of view about preservation of different bones.

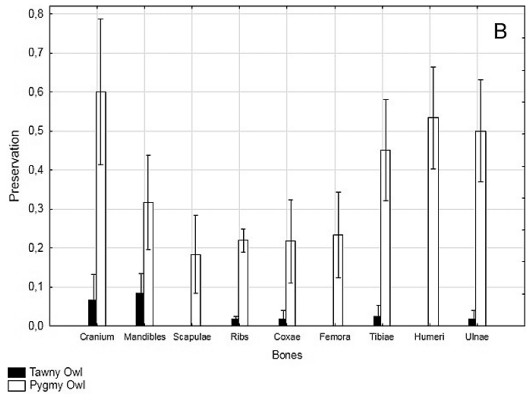
Studies of the preservation of skeletal elements in natural conditions is very difficult because the initial characteristics of the prey are unknown. However an experiment in laboratory conditions allows estimating morphometric and age parameters of prey and making the analysis of preservation more effective and exact. Therefore, the aim of our research was to investigate: (1) which skeletal elements of prey have the highest preservation in owl pellets; (2) whether there are differences in the digestibility of the skeletal elements of the prey in the pellets of three individuals of three owl species; (3) whether there are differences in the digestibility of the skeletal elements of the different age classes of prey.

2. Material and methods

The laboratory research was done with three individuals of three different species: Long-eared Owl (*Asio otus*), Tawny Owl (*Strix aluco)* and Pygmy Owl (*Glaucidium passerinum*). The individuals were all males. Adult owls were kept in laboratory conditions and fed on mice (*Mus musculus*). Owl pellets were collected daily and examined using a standard technique (Marti 1987). After that, we counted the number of preserved bones and estimated their preservation. The preservation of bones was defined as a ratio of the number of whole specimens of a certain bone to the total number of bones which were contained in the body of one mouse (Potapov 1989).

During the experiment we gave 60 adult mice (age was 1,5–4 months) to the Long-eared Owl. The owl was fed one mouse per day. We gave mice to Tawny owl in two stages: first stage – 60 adult individuals, second stage – 60 immature individuals (aged 3–4 weeks). The owl got two adult mice per day and once a week we did not feed the bird (fasting day). Otherwise the mice were not eaten, or partially eaten. In the second phase we first gave two immature individuals, but pellets were not formed and the owl starved. Therefore, during a second experiment we increased the number to 4–5 individuals per day (this number corresponds to approximately two adult mice by weight). We





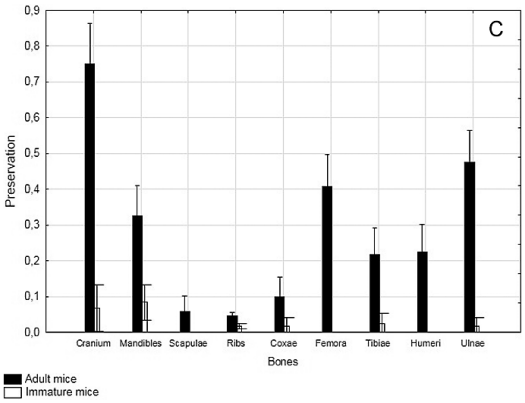


Fig. 1. The preservation of bones of mice of different ages in the pellets of owls: (A) adult mice; (B) immature mice; (C) adult and immature mice in the pellets of Tawny Owl.

gave 30 immature mice to the Pygmy Owl. In the beginning of the experiment, we fed the Pygmy Owl adult mice, but it ate the adult mice only partly. The hind part of the adult mice was not eaten and we conducted the experiment only with immature mice.

The preservation of mice bones was studied using a General Linear Mixed Model (GLMM) with logit link function and binomial error distribution (Zuur *et al*. 2009). To fit the model we used the lme4 package (Bates *et al*. 2016) in R (version 2.15.21; R Core Team 2012). For the analysis of preservation of skeletal elements of prey in owl pellets, the preservation of mice bones in the pellets of three species of owls was used as a dependent variable in the model. The type of bones, age of mice and species of owl were used as independent variables. Also, in addition to the above independent variables, the model contained a random effect – the serial number of prey. The influence of the random effect on the dependent variable was recognized insignificant in the model. Analysis of skeletal elements in the pellets of individuals of three species of owls showed that the upper part of the cranium had the highest preservation in most cases (Fig. 1). In connection with this, further analysis of the preservation of other skeletal elements was carried out relative to the preservation of the upper part of the cranium (Table 1). We made three models which allow comparing the preservation of: (1) skeletal elements of adult mice in the pellets of Long-eared Owl and Tawny Owl; (2) skeletal elements of immature mice in the pellets of Pigmy Owl and Tawny Owl; (3) skeletal elements of adult and immature mice in the pellets of Tawny Owl. In general the preservation of 4920 elements was analyzed.

3. Results

The upper part of cranium, femora, mandibles, ribs, coxae and humeri had the highest preservation (i.e. more than 90%) among skeletal elements

Table 1. Estimated effects and their standard errors (SE), from the model containing all variables. All numbers indicate statistically significant effects ($p < 0.05$), except ns where $p = 0.09$.

Parameter	Estimate	SE
Adult mice (Long-eared Owl & Tawny Owl's pellets)[1]		
Intercept	9.09	0.53
Femora	–2.37	0.45
Mandibles	–3.31	0.47
Ulnae	–3.44	0.47
Humeri	–4.17	0.49
Coxae	–5.40	0.52
Ribs	–6.05	0.44
Tibiae	–9.13	0.50
Scapulae	–10.27	0.52
Compare of total preservation of bones in the Long-eared Owl pellets and Tawny Owl pellets[2]	–7.06	0.43
Immature mice (Tawny Owl &Pygmy Owl's pellets)[1]		
Intercept	–6.24	1.98
Femora	–9.36	1.50
Mandibles	–3.22	0.98
Ulnae	–2.80	0.96
Humeri	–2.42	0.95
Coxae	–8.56	1.46
Ribs	–8.48	1.09
Tibiae	–3.07	0.97
Scapulae	–14.49	2.76
Compare of preservation of bones in the Pygmy Owl pellets and Tawny Owl pellets[2,3]	–4.10 ns	2.42 ns
Adult & immature mice (Tawny Owl's pellets)		
Intercept	2.27	0.63
Femora	–2.93	0.48
Mandibles	–2.83	0.47
Ulnae	–2.21	0.45
Humeri	–4.76	0.55
Coxae	–6.63	0.67
Ribs	–8.92	0.60
Tibiae	–4.57	0.54
Scapulae	–9.11	0.95
Compare of preservation of adult mice bones and immature mice bones	–7.44	0.97

1) The preservation of other skeletal elements was carried out relatively the preservation of the upper part of the cranium without species separation.
2) The differences in the preservation of skeletal elements in the pellets of different owl species was carried out without separation by type of bones.
3) P = 0.09 for analysis of differences in bones preservation in the pellets of Pygmy Owl and Tawny Owl.

of adult mice in the pellets of Long-eared Owl. Furthermore the preservation of the upper part of cranium and femora was 100%. In addition, the preservation of ulnae was high (75.5%) (Fig. 1, A). The upper part of the cranium, ulnae and femora prevailed among preserved bones in the pellets of Tawny Owl. The upper part of the cranium had the highest preservation among these bones (Fig. 1, A).

The mandibles had the highest preservation among the skeletal elements of immature mice in Tawny Owl pellets. However, the preservation of

bones of immature mice was very low and did not exceed 10%. Furthermore, the preservation of the upper part of cranium was lower than the preservation of mandibles. The upper part of the cranium, humeri, ulnae and femora had the highest preservation among skeletal elements of immature mice in the pellets of Pygmy Owl. The upper part of the cranium had the highest preservation among these bones (60%) (Fig. 1, B).

In general, we noticed that the upper part of the cranium practically had higher preservation than all other bones. It concerned the preservation of bones of adult and immature mice. The preservation of mandibles of immature mice in the pellets of Tawny Owl was a little lower than the preservation of the upper part of cranium. However, these differences were not statistically significant. Moreover, femora, mandibles, ulnae and humeri had high preservation among skeletal elements of adult mice in the pellets of Long-eared Owl and Tawny Owl. The femora, ulnae, tibiae and mandibles had high preservation among skeletal elements of immature mice in the pellets of Pygmy Owl and Tawny Owl (Fig. 1).

According to modelling results, the preservation of the skeletal elements of mice differed in the pellets of three individuals of three species of owl. The preservation of skeletal elements of adult mice in the pellets of Tawny Owl was lower than in the pellets of Long-eared Owl (Table 1). Moreover, preservation of bones of immature mice tended to be lower ($p = 0.09$, Table 1) in the pellets of the Tawny Owl than in the pellets of the Pygmy Owl.

The comparative analysis of the preservation of skeletal elements of adult and immature mice in the pellets of Tawny Owl showed that the skeletal elements of adult mice had higher preservation than the skeletal elements of immature mice (Table 1, Fig. 1).

4. Discussion

Almost all skeletal elements of mice had lower preservation than the upper part of cranium in the pellets of three individuals of different species of owls. Similar data on preservation of the upper part of cranium in the pellets of owls were obtained by Lowe (1980). In the laboratory conditions, the author found that the preservation of the upper part of cranium of prey in the pellets of Tawny Owl was 81%. According to our results, this value was 75% (Fig. 1).

Nevertheless, the preservation of some skeletal elements of postcranial skeleton of adult and immature mice was high (Fig.). Femora, ulnae and humeri had relatively high preservation. The high preservation of these skeletal elements has been marked by other authors (Raczyński & Ruprecht 1974, Potapov 1989, Priklonskii & Ivanchev 1993). Moreover, some studies showed that humeri, radii and tibiae of small mammal skeletons had high preservation in the pellets (Potapov 1989, Mitropolsky & Fundukchiev, 2009). In fact, the humeri of mice had high preservation in the pellets of the Long-eared Owl. Mitropolsky & Mitropolsky (2009) provided similar data on the preservation of humeri in the pellets of Long-eared Owl. According to their results, the preservation of humeri was 88.8% and exceeded preservation of the cranium. Meanwhile, the preservation of humeri in the pellets of the studied individual of Tawny Owl was lower – 22.3%. However, our study did not show very high preservation of tibiae. According to our results, tibiae had low preservation in the pellets of Long-eared Owl and Tawny Owl. Moreover, we did not notice radii in the pellets of owls. Other researchers showed that coxae, scapulae and ribs have low preservation in the pellets of prey birds (Potapov 1989).

In our study coxae and ribs had a high preservation in the pellets of Long-eared Owl but low preservation in the pellets of Tawny Owl and Pygmy Owl. The scapulae had low preservation in the pellets of all three studied individuals of owls.

The preservation of skeletal elements of adult mice was higher in the pellets of Long-eared Owl than in the pellets of Tawny Owl. Differences in the preservation of skeletal elements in the pellets of Long-eared Owl, Barn Owl and Tawny Owl were shown earlier by Raczyński & Ruprecht (1974) who found that the highest total preservation of the bones of cranium and coxae was observed in the pellets of Barn Owl (65.8%). The total preservation of these bones in the pellets of Long-eared Owl was 54.1%, while a lower preservation was found in the pellets of Tawny Owl (49.2%). However, these data were based on pellets of immature birds while we analyzed pellets of adult owls. According to our results, the preserva-

tion of these skeletal elements in the pellets of Long-eared Owl was 95.8%. The total preservation of the bones of cranium and coxae was not high in the pellets of Tawny Owl (39.2%). In both studies the total preservation of these bones was higher in the pellets of Long-eared Owl than in Tawny Owl. Moreover, we showed here that there seem to be differences in preservation of immature mice in the pellets of Pygmy Owl and Tawny Owl. Notably, the preservation of bones was lower in the pellets of Tawny Owl. We suppose that the difference in the preservation of bones in the pellets of three individuals of owls depends on the features of digestion of owls. However, this question requires more detailed study.

In Tawny Owl pellets the preservation of skeletal elements of immature mice was lower than the preservation of bones of adult mice. These differences were clear for preservation of the upper part of cranium, which was 75% in adult and 6.7% in immature mice. The similar results were shown by Raczyński & Ruprecht (1974). The preservation of bones of immature mice was 2–4 times lower, than the preservation of bones of adult animals. We consider that the cause of these is the age differences in the size and thickness of skeletal elements of mice. The skeletal elements of immature mice are smaller and thinner than bones of adult animals. Therefore they are digested to a higher degree.

We conclude that it is not always true that identification based on postcranial skeletal elements is less effective. Some large elements of the postcranial skeleton are not less well preserved than the cranial elements in the pellets. For example, the preservation of some large elements of the postcranial skeleton was high in the analysis of Long-eared Owl pellets. In general, the largest and most massive bones have the highest preservation.

Studies and analyses of postcranial elements of prey may allow to resolve additional objectives. For example, sexual dimorphism of coxae allows determining the sex ratio of the prey (Brown & Twigg 1969, Mayakov & Shepel 1987). In addition, we are used to consider that birds of prey eat head of prey first. However, sometimes there is a situation where a predator cannot eat a large prey whole, and eats only its hind part (Mitropolsky & Mitropolsky 2009). In this case, it is advisable to use postcranial skeletal elements.

Based on literature and our own data, we consider that prey identification methods based on cranial and postcranial elements complement each other. We therefore advise to use both elements together for maximum reliability of estimations of species composition and the number of prey based on pellet analysis.

Acknowledgements. We are grateful to A. Goreva for assistance in the laboratory studies.

Jyrsijöiden luiden jäänteet Strigiformes -suvun pöllöjen pelleteissä: vertaileva laboratoriotutkimus

Pöllöjen ravintokohteita voidaan selvittää luiden jäänteistä oksennuspelleteissä. Jotta voitaisiin luotettavasti selvittää ravintoa luiden avulla, täytyy ymmärtää miten eri luut säilyvät pelleteissä useilla pöllölajeilla.

Tässä tutkimuksessa selvitettiin kraniaalisten ja postkraniaalisten luiden säilymistä, käyttäen hyväksi laboratoriohiiriä. Luiden säilymistä tutkittiin sarvipöllön, lehtopöllön ja varpuspöllön pelleteistä (kolme koirasyksilöä). Pöllöille syötettiin aikuisia ja nuoria hiiriä, ja eri luiden säilymistä pelleteissä seurattiin. Suurimmat luut, kallon yläosa ja raajojen isot luut, säilyivät parhaimmin pelleteissä. Täysikasvuisen hiiren luut säilyivät parhaiten sarvipöllön pelleteissä. Nuoren hiirten luut säilyivät paremmin varpuspöllön kuin sarvi- tai helmipöllön pelleteissä. Nuorilla hiirillä on pienemmät ja ohuemmat luut kuin aikuisella, mikä todennäköisesti selittää eroja eri ikäisten hiirten luiden säilyvyydessä.

Aiemmat tutkimustulokset ja tämän tutkimuksen löydökset viittaavat siihen, että luotettavimmat tulokset pöllöjen saaliseläimistä ja niiden määrästä saadaan kun analysoidaan sekä kraniaalisia että postkraniaalisia luita.

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